



# Utilizing Superoxide Dismutase Mimetics to Enhance Radiation Therapy Response While Protecting Normal Tissues

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Symptomatic normal tissue injury is a common side effect following definitive therapeutic radiation and chemotherapy treatment for a variety of malignancies. These cancer therapy related toxicities may occur acutely during treatment resulting in reduced or missed therapy agent administration or after the completion of therapy resulting in significant chronic morbidities that significantly diminish patient quality of life. Radiation and chemotherapy induce the formation of reactive oxygen species (ROS) both in normal tissues and tumor cells. One type of ROS common to both chemotherapy and radiation therapy is the formation of superoxide ( $O_2^{\bullet-}$ ). Fortunately, due to metabolic differences between cancer and normal cell metabolism, as well as improved targeting techniques, ROS generation following radiation and chemotherapy is generally greater in cancer cells compared to normal tissues. However, the levels of ROS generated in normal tissues are capable of inducing significant toxicity. Thus, several groups are focusing on metabolism-based approaches to mitigate normal tissue effects occurring both during and following cancer therapy. This review will summarize the most current preclinical and clinical data available demonstrating the efficacy of small molecule, superoxide dismutase mimetics in minimizing radiation and chemotherapy-induced normal tissue injury, resulting in enhanced patient outcomes. *Semin Radiat Oncol* 29:72–80 Published by Elsevier Inc.

## Introduction

Reactive oxygen species (ROS) generated under physiological conditions serve an essential role as signaling molecules in cell processes ranging from cell proliferation to apoptosis. However, when generated under nonphysiological, stress-related conditions, such as during therapeutic radiation and chemotherapy administration, ROS becomes toxic both to

tumor cells and surrounding normal tissue.<sup>1-3</sup> Radiation and chemotherapy associated normal tissue toxicities may occur acutely during treatment as well as persisting months to years after radiation and chemotherapy are completed. The toxicities from cancer therapies cause changes in mitochondrial metabolism that lead to formation of several ROS.

Superoxide ( $O_2^{\bullet-}$ ) is a critical ROS formed during cancer therapy. At physiological concentrations,  $O_2^{\bullet-}$  is essential to

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normal signaling processes but with increased cellular concentrations induces toxicity. Physiological levels of  $O_2^{\bullet-}$  are generated during metabolic respiration, and by the activity of cytochrome p450 enzyme isoforms, alpha-ketoglutarate dehydrogenase, glycerol-3-phosphate dehydrogenase, and NADPH-dependent oxidases.<sup>4</sup> When cells are stressed, more  $O_2^{\bullet-}$  is generated, as is observed during and following treatment with anti-cancer therapies, including chemotherapy<sup>5</sup> and radiation therapy.<sup>6,7</sup> Superoxide dismutase enzymes (SODs) convert  $O_2^{\bullet-}$  to hydrogen peroxide ( $H_2O_2$ ). The  $H_2O_2$  is subsequently converted to  $H_2O$  and  $O_2$  by catalase or other peroxidases.

During radiation and chemotherapy, the cooperation between SOD enzymes and peroxidases are not in balance resulting in oxidative damage. In the past 30 years, several groups have developed and utilized small molecule superoxide dismutase mimetics that are administered prior to and/or during radiation and chemotherapy to minimize increased steady-state levels of  $O_2^{\bullet-}$  in order to mitigate normal tissue injury. The following is a brief review of 2 such compounds: Galera Compound 4419 (GC4419) and Mn porphyrin-based SOD mimics (Fig. 1).

### GC4419 (Formerly Known as M40419)

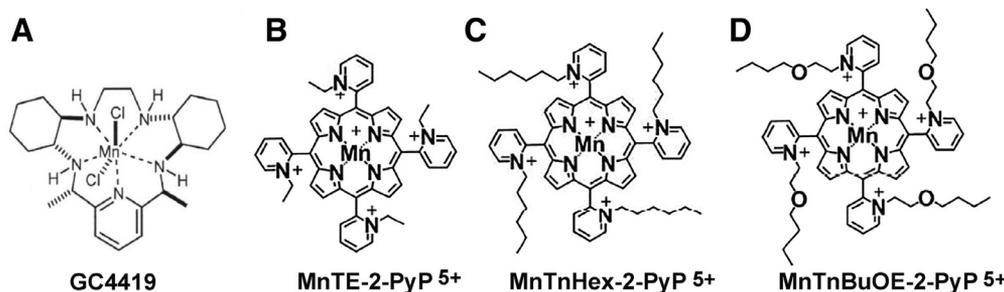
GC4419 is a member of the pentaaza-macrocyclic Mn(II) class of SOD mimetics that catalyzes the dismutation of  $O_2^{\bullet-}$  into  $H_2O_2$  at a rate constant of  $2 \times 10^7 M^{-1} s^{-1}$ , which is comparable to the native SOD enzyme ( $2 \times 10^9 M^{-1} s^{-1}$ ).<sup>8,9</sup> GC4419 selectively removes superoxide without reacting with other reactive oxygen species, including nitric oxide, hydrogen peroxide, and peroxyxynitrite. The protective effects of GC4419 or an active enantiomer (GC4403 formerly known as M40403) against radiation-induced toxicity in cancer but not normal cells have been demonstrated in vitro as well as in animal models.<sup>10-12</sup> The selective protection GC4419 for normal and not cancer cells is thought to arise because of fundamental differences in cancer cell mitochondrial oxidative metabolism leading to greater steady-state levels of  $O_2^{\bullet-}$ , which in turn leads to selective increases in steady-state levels of  $H_2O_2$  in cancer cells treated with GC4419 and not normal cells.<sup>13</sup> Furthermore, GC4419 has been shown to reduce chemoradiation-induced mucositis in clinical trials.<sup>14</sup>

### Mitigating Oral Mucositis

Oral mucositis (OM) is a common side effect of chemoradiation for head and neck squamous cell carcinoma that is painful, disruptive and difficult to manage.<sup>15</sup> Approximately 70% of patients receiving concurrent cisplatin and radiation for oral cavity and oropharynx cancer will experience severe oral mucositis (SOM).<sup>16,17</sup> The median time to development of SOM is the fourth week of a 6–7-week treatment course and the median duration is 3–4 weeks. Patients who suffer this painful complication commonly require narcotic analgesics and other medications for pain. OM adversely affects nutrition, hydration, speech, swallowing, quality of life, bacteremia risk, and feeding tube placement and use rates, leading to increased hospitalization, emergency room use, and healthcare costs.<sup>18-21</sup> Importantly, SOM is also associated with chemoradiation treatment breaks, which is known to impact tumor control rates in head and neck cancer.<sup>22,23</sup>

Evidence-based management of OM is limited to palliation and pain control with topical agents and systemic analgesics.<sup>24-32</sup> The only FDA-approved drug to reduce OM, palifermin, is a keratinocyte growth factor that is indicated for mitigating OM only in patients with hematologic malignancies receiving myelotoxic therapy requiring hematologic stem cell support.<sup>16,17,33</sup> Therefore, there is an urgent need to mitigate OM and several drugs are currently in Phase II/III testing.<sup>34</sup>

A Phase 1b/2a trial tested GC4419 in combination with intensity-modulated radiation (IMRT) and concurrent cisplatin (either 100 mg/m<sup>2</sup> every 3 weeks or 30–40 mg/m<sup>2</sup> weekly) in patients with American Joint Committee on Cancer version 7 Stage III–IVb oral cavity and oropharynx squamous cell carcinoma.<sup>14</sup> The IMRT plan had to include at least 2 oral mucosal sites (right or left buccal mucosa, right or left ventral/lateral oral tongue, floor of mouth, or soft palate) within the 50 Gy cumulative isodose line. GC4419 was delivered as a 60-minute IV infusion, ending within 60 minutes prior to each radiation fraction. OM was assessed by trained investigator-evaluators using the World Health Organization (WHO) criteria, in which: Grade 0 = No mucositis; Grade 1 = Pain and erythema; Grade 2 = Ulceration, able to eat solid food; Grade 3 = Ulceration, able to eat only liquids; and Grade 4 = Ulceration, inability to eat requiring tube or parenteral feeding. By the WHO criteria, SOM is defined as either Grade 3 or Grade 4 OM. OM was assessed twice



weekly with at least a 48-hour interval between assessments during IMRT and weekly thereafter for up to 8 weeks or until the WHO score was  $< 2$ . The primary endpoint was to determine safety of dose and schedule escalation concurrent with cisplatin/RT. Secondary endpoints included efficacy of the drug in delaying onset of SOM, decreasing severity and duration of SOM.

The study followed a serial cohort dose-escalation design with 3-6 patients per cohort, testing 15, 30, 50, 75, and then 112 mg of GC4419 per dose delivered before each of the first 14 IMRT fractions. Based on observed safety results in these first 5 cohorts, the protocol was amended to allow testing "duration extension" cohorts of 112 mg of GC4419 prior to each RT fraction in weeks 4, 5, and 6. Subsequent review of safety and OM results through these cohorts led the sponsor to add 3 cohorts to extend dosing further at reduced doses: 90 mg for 6 or 7 weeks, or 30 mg for 7 weeks.

Nine US centers enrolled 46 patients, the majority of whom were male with stage IVa oropharyngeal cancer treated with definitive high-dose cisplatin with 70 Gy IMRT. 41 of 46 patients (89%) received all planned GC4419 infusions. Observed safety was acceptable at all dose and duration schedules studied and delivery of planned chemoradiotherapy was not compromised in the presence of GC4419. Grade 3 nausea was the drug-related dose-limiting toxicity at 112 mg, but because no single cohort had  $> 1$  patient with dose-limiting, the maximum tolerated dose was not considered exceeded. Grade 3 nausea was the reason for dose reduction to 90 mg for subsequent duration extension cohorts. Higher doses of GC4419 also resulted in increased incidence of a transient, infusion-related Grade 1 facial paresthesia that spontaneously resolved shortly following the infusion.

The incidence, duration, and time to onset of SOM appeared improved with GC4419 compared to historical controls. For patients who received 30 mg or 90 mg of GC4419 over the full 6-7 weeks of CRT, the cumulative incidence of SOM was 29% (4 of 14) through 6 weeks of RT (60 Gy), and 50% (7 of 14) at any time, with a median time to onset of  $> 50$  days, and a median duration of 2.5 days. Tumor control at 1 year was not compromised.

These promising results led to the design of a randomized, double-blind, and placebo-controlled Phase 2b trial testing placebo vs 30 mg or 90 mg of GC4419 prior to every RT fraction in the same patient population.<sup>35</sup> A total of 44 US and Canadian institutions enrolled 223 oral cavity and oropharynx cancer patients who were randomized 1:1:1 and stratified prospectively by human papillomavirus status and cisplatin regimen (weekly vs q3 week). The primary endpoint of duration of SOM was evaluated by intention to treat analysis, with patients who did not experience SOM assigned a duration of 0 days. Patients in the placebo arm had a median 19-day duration of SOM, compared with 1.5 days in patients who received the 90-mg dose of GC4419, a 92% relative reduction ( $P = 0.024$ , unpublished data). Patients in the 90-mg arm demonstrated improvements in incidence of SOM (43% vs 65%,  $P = 0.009$ ), the incidence of grade 4 mucositis (16% vs 30%,  $P = 0.045$ ), and time to onset (median 61 vs 39 days). There were intermediate

improvements seen in the 30-mg arm. Safety was comparable across all three arms and GC4419 side effects (ie, hypotension and oral/facial paresthesia) were mild and transient, usually resolving within 1 hour of infusion completion. The Food and Drug Administration recently granted Breakthrough Therapy and Fast Track designations to GC4419 (Galera Therapeutics, Inc. Press Release, February 28, 2018) for the reduction of the severity and incidence of radiation and chemotherapy-induced oral mucositis. A Phase 3 randomized trial of 90 mg of GC4419 vs placebo is scheduled to begin before the end of 2018.

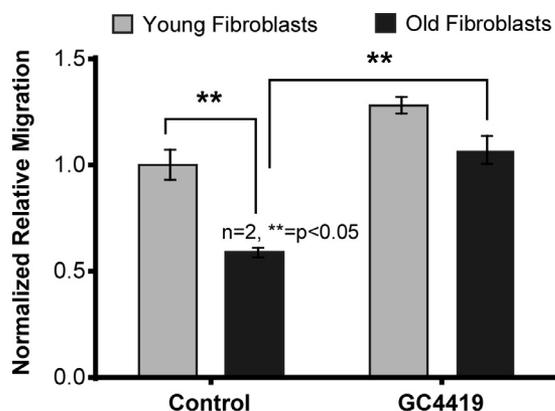
## GC4419 and Aging

Dysregulated expression of genes that encode for mitochondrial electron transport proteins accumulate with aging, resulting in increased leakage of electrons in the mitochondrial electron transport chain and enhanced steady-state levels of  $O_2^{\bullet-}$ .<sup>36-38</sup> This age associated increase in  $O_2^{\bullet-}$  makes elderly normal tissues more susceptible to toxicity incurred by radiation and chemotherapy.<sup>10</sup> Mapuskar et al demonstrated that treatment of fibroblasts from elderly patients with GC4419 prior to exposure of therapeutic radiation doses or platinum-based chemotherapy prevented much of the enhanced toxicity associated with aging.<sup>10</sup> Furthermore, treatment with GC4419 was able to partially restore mitochondrial activity and function without interfering with therapeutic radiation or chemotherapy-induced cancer cell killing.<sup>10</sup>

Another critical facet necessary for wound healing induced from therapeutic radiation and chemotherapy is the migration of healthy fibroblasts to the injury site. Fibroblasts collected from elderly individuals ( $\geq 70$  years old) have reduced migrating ability compared to fibroblasts from young individuals ( $\leq 2$  years old, Fig. 2). Moreover, treatment of the fibroblasts collected from elderly patients with GC4419 restored the migration ability to that of fibroblasts collected from young patients (Fig. 2). Thus, SOD mimics may prove to be even more beneficial in protecting from toxicities resulting from cancer treatment in elderly populations.

## Mn Porphyrin-Based SOD Mimics

One of the earliest made Mn porphyrin (MnPs) was Mn (III) *ortho* substituted *N*-pyridyl compound, Mn (III) *meso*-tetrakis (*N*-ethylpyridinium-2-yl) porphyrin, MnTE-2-PyP<sup>5+</sup> (AEOL10113, BMX-010, MnE, Fig. 1).<sup>39</sup> The *ortho* positioned cationic pyridyl nitrogens are close to the Mn center and impose a strong electron-withdrawing effect that results in thermodynamic properties close to those of native SOD enzymes. The penta-positive charge of MnE promotes favorable electrostatics by attracting the negatively charged superoxide, mimicking the SOD enzyme's kinetics.<sup>40</sup> Although MnE was shown to be an effective superoxide scavenger in many models, its excessive hydrophilicity inhibited penetration across the blood brain barrier.<sup>41</sup> Therefore, new drugs were designed and among these were Mn(III) *meso*-tetrakis (*N*-n-hexylpyridinium-2-yl) porphyrin, MnTnHex-PyP<sup>5+</sup> (MnHex) and Mn(III) *meso*-tetrakis (*N*-n-butoxyethylpyridinium-2-yl)porphyrin, MnTnBuOE-2-PyP<sup>5+</sup> (BMX-001,



**Figure 2** GC4419 restores the migration ability of fibroblasts collected from elderly individuals to that of the ability of fibroblasts from young individuals. Exponentially growing young (5-month-old foreskin) and old (78-year-old male) human dermal fibroblasts were seeded in fibroblast basal media with growth factors. Cells were pre-treated for 24 hours with GC4419 (0.25  $\mu$ M). Invading fibroblasts were stained using Thiazolyl Blue Tetrazolium Bromide (MTT) solution. GC4419 was able to restore the migration ability of fibroblasts from the 78-year-old male to that of a 5-month-old male.

MnBuOE, Fig. 1). MnHex and MnBuOE are  $\sim$ 4000-fold more lipophilic than MnE,<sup>42-44</sup> and both MnHex and MnBuOE were able to cross the blood brain barrier and protect the brain against oxidative damage.<sup>45</sup>

MnPs are reactive not only with superoxide but with numerous small and large reactive oxygen, nitrogen, and sulfur species.<sup>46</sup> MnPs have catalase and glutathione peroxidase like activities, peroxynitrite-reducing activity and the ability to oxidize ascorbate.<sup>42</sup> MnPs act equally as anti-oxidants (reducing  $O_2^{\bullet-}$  to  $H_2O_2$ ) and pro-oxidants (oxidizing  $O_2^{\bullet-}$  to  $O_2$ ). If  $H_2O_2$  is maintained under nM levels, as is the case in normal cells, the catalysis of  $O_2^{\bullet-}$  dismutation results in antioxidant therapeutic effects. Reactions with other molecules besides superoxide can result in MnPs acting as either anti-oxidants or pro-oxidants.

## Mn Porphyrins as Radioprotectors

MnPs have been used in a variety of models of radiation by many labs, which will be discussed in detail in the following paragraphs. MnPs protect from gamma, x-ray, and proton beam irradiation. The SOD mimics can protect from 1 localized large dose of radiation or smaller fractionated doses of radiation. In most cases, MnP is given before radiation exposure and continued after radiation exposure. However, a few studies indicate that beginning treatment with MnP after radiation exposure may also prevent radiation-induced injury. These preclinical studies are discussed in further detail below.

The very first radiation studies with MnP demonstrated that MnE is a potent radioprotector by inhibiting TGF- $\beta$  signaling and collagen deposition, resulting in the protection of lung tissues from radiation damage.<sup>47</sup> These

data have been confirmed in several subsequent studies showing that MnE inhibits radiation-induced damage in rodent lung tissues when MnE is given prior to radiation.<sup>48-50</sup> Radiation protection has also been observed when administration of MnPs began 14 days after radiation.<sup>49</sup> In addition, MnTnHex-2-PyP was effective in protecting irradiated lungs from nonhuman primates when given 2 hours after RT.<sup>51</sup>

The GI tract is another organ that is highly sensitive to radiation. Radiation-induced proctitis is a common concern when irradiating tumors in the pelvic region. Archambeau et al irradiated a small segment of the rectum with 20-30 Gy protons.<sup>52</sup> MnE was given via i.p. injection 1 hour before radiation or 1 hour after radiation and then given weekly for the rest of the experiment. In these models, protection was only observed when MnE was given before radiation. In an acute injury model, by day 10 postradiation the control group suffered from significant crypt loss and MnE protected from crypt cell death. In a chronic model, MnE prevented rectal dilation and proctitis. At 9 months postradiation, 80% of the irradiated alone mice developed proctitis, while only 20% of mice developed proctitis in the group that received MnE prior to radiation.<sup>52</sup> Thus, MnE treatment greatly protected the rectal tissues from acute and chronic radiation-induced damage.

Pelvic irradiation is used frequently to treat prostate cancer and unwanted side effects can arise due to radiation exposure, such as erectile dysfunction and bladder incontinence. Rats were irradiated with 5 fractionated doses of 7.5 Gy to the pelvis and MnE was given 24 hours before radiation and then dosed after radiation for 12 weeks. Treatment of rats with MnE protected animals from erectile dysfunction after radiation.<sup>53</sup> During pelvic irradiation, the addition of MnE was also found to protect from skin and bladder fibrosis.<sup>54</sup>

MnBuOE protected salivary glands from a single high dose of radiation to the head and neck area when injected twice daily for the duration of study.<sup>55</sup> These mice were irradiated with single doses up to 15 Gy and treatment with MnBuOE resulted in less inflammation in the irradiated area and protected from saliva loss due to radiation exposure.<sup>55</sup> The dose-modifying factor for the protection against xerostomia/salivation was 0.77. The study was repeated using  $\sim$ one tenth the dose of MnBuOE and the lower dose of MnBuOE still protected against oral mucositis, xerostomia, and salivary gland fibrosis.

Since MnBuOE crosses the blood brain barrier, this drug was also tested for its ability to protect brain tissues from radiation damage.<sup>45,56</sup> Protection of neurogenesis was seen when MnBuOE was given sc daily for a week prior to cranial irradiation (5 Gy) and a week following radiation. MnBuOE supported production and long-term survival of newborn neurons in the hippocampal dentate.<sup>45</sup> In other study, MnBuOE was given before and after whole brain radiation (8-10 Gy) and MnBuOE protected the loss of white matter, specifically loss in axons and myelin in the corpus callosum.<sup>57</sup> The addition of MnBuOE also maintained rotarod running performance

in irradiated mice.<sup>58</sup> Thus, lipophilic SOD mimics protect both tissue integrity and neuronal function following brain irradiation.

## Mechanisms of MnP as Radioprotectors

### MnPs Regulate Immune Response

The first in-depth mechanism described for MnE was its ability to inhibit NF- $\kappa$ B signaling in macrophages. Although these studies did not include radiation, they illustrate that these SOD mimics inhibit inflammation, which plays a role in radiation injury. Piganelli's work suggests that MnE oxidizes the p50 subunit of NF- $\kappa$ B.<sup>59</sup> The oxidized p50 no longer binds to DNA, making NF- $\kappa$ B inactive and unable to transcribe proinflammatory genes in macrophages.<sup>59</sup> These findings have also been validated in a variety of ischemia/reperfusion models demonstrating that MnP can prevent injury due to ischemia/reperfusion, in part, by inhibiting NF- $\kappa$ B signaling.<sup>60,61</sup>

### MnPs Inhibit TGF- $\beta$ Signaling

During lung radiation, MnE treatment reduced serum TGF- $\beta$  levels in mice.<sup>47</sup> In subsequent studies conducted in irradiated, hypoxic macrophages, it was demonstrated that MnE treatment reduced levels of superoxide, TGF- $\beta$  and VEGF levels in these cells.<sup>62</sup> MnHex also reduced TGF- $\beta$ , HIF-1 $\alpha$ , and VEGF signaling in irradiated lungs.<sup>50</sup> In addition, MnHex inhibited macrophage recruitment and reduced DNA oxidative damage in the irradiated lungs. Therefore, there is solid evidence that MnP treatment results in the reduction of TGF- $\beta$  signaling and inflammation in the irradiated lung.

The fibroblast is the cell most responsive to TGF- $\beta$ . In the presence of TGF- $\beta$ , a fibroblast will differentiate into a myofibroblast, which is the cell type responsible for laying down aberrant extracellular matrix. Using an in vitro fibroblast model, MnE or MnBuOE treatment prevented the activation of regular fibroblasts into myofibroblasts in response to radiation.<sup>63,64</sup> In vivo skin from irradiated mice produce elevated numbers of fibroblasts expressing  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), which is a marker for activated myofibroblasts. In contrast, in irradiated, MnE treated mice, no elevation of  $\alpha$ -SMA was observed in the skin of these animals.<sup>54</sup> Thus, indicating that MnP treatment inhibits the activation of fibroblasts and, thus, fibrosis.

A more in-depth investigation of the TGF- $\beta$  signaling pathway revealed that MnE treatment resulted in a small reduction in active TGF- $\beta$ 1 levels, a large reduction of TGF- $\beta$ RII protein levels (the receptor for active TGF- $\beta$ 1 levels) and a reduction in SMAD/phosphorylated SMAD protein levels (transcription factors that transcribe fibrotic genes).<sup>63</sup> It was also demonstrated that plasminogen activator inhibitor-1 (PAI-1), a target of the TGF- $\beta$ /SMAD pathway, was also reduced with MnE treatment.<sup>63</sup> Further studies have also demonstrated that collagen genes, also controlled by the

TGF- $\beta$ /SMAD, are similarly reduced with MnE treatment.<sup>54</sup> Thus, MnE treatment results in the reduction of the entire TGF- $\beta$ /SMAD signaling pathway in fibroblasts in response to radiation.

### MnPs Enhance Cytoprotective Pathways

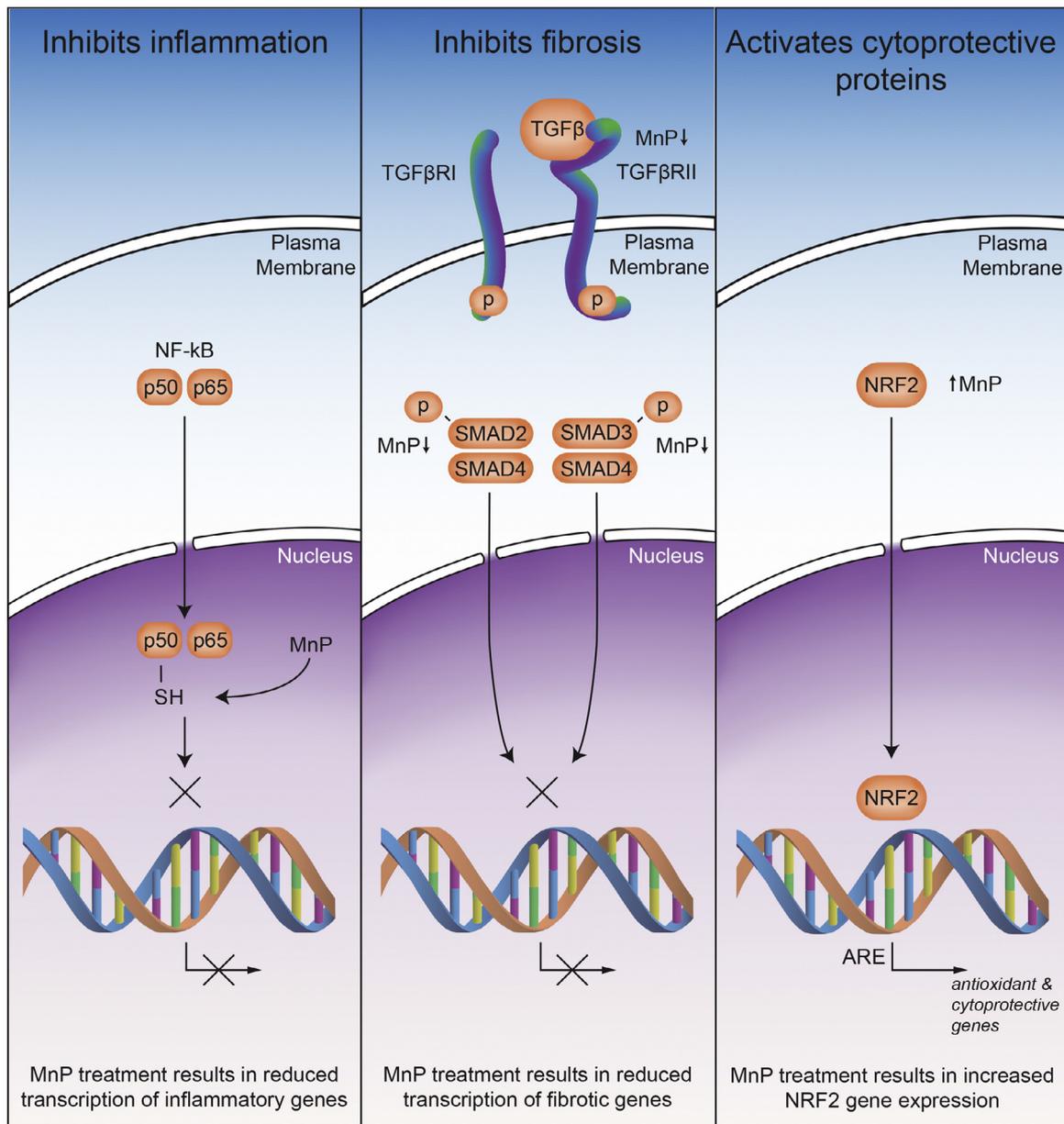
In addition to inhibiting inflammation and TGF- $\beta$  signaling, MnPs have also been shown to activate the nuclear factor erythroid 2-related factor 2 (NRF2) transcription factor.<sup>65</sup> NRF2 activation can be regulated by oxidation of redox-sensitive Keap1; however, it is unclear if this is the only mechanism(s) by which MnPs are regulating NRF2 at this time. NRF2 upregulates a variety of antioxidant and cytoprotective genes. In a recent report, MnBuOE treatment resulted in the activation of NRF2 transcription factor, which resulted in the increased transcription of NADPH quinone dehydrogenase 1 (NQO1) mRNA and an increase in MnSOD, catalase and other antioxidant genes in hematopoietic stem cells.<sup>65</sup>

In another recent report, NQO1 mRNA and protein levels were shown to be highly upregulated with MnE treatment in fibroblasts and these levels were enhanced when MnE treatment was combined with radiation.<sup>54</sup> Thus, MnP treatment may protect normal cells from radiation damage by upregulating NRF2 signaling and enhancing the expression of antioxidant and cytoprotective proteins. MnP treatment seems to boost the cell's natural cytoprotective machinery to inhibit damage due to radiation exposure.

In summary, in normal tissue injury, MnPs suppress oxidative stress by reducing levels of reactive species. Such effects may be due, in part, to the direct removal of reactive species. Yet, more likely, MnPs inhibit those pathways that give rise to reactive species, which promote detrimental effects to the cells (Fig. 3). One way in which MnP regulates signaling is through the oxidation of key cysteines of redox sensitive proteins.<sup>42</sup> The 3 major pathways identified, NF- $\kappa$ B, TGF- $\beta$ , and NRF2, are all regulated through oxidation of cysteines. There is also potential cross talk between the pathways that could result in the amplification of the changes induced by MnP treatment. However, more work is needed to fully understand how MnP are reducing radiation damage in normal cells.

## Mn Porphyrins as Anticancer Drugs

MnE or MnBuOE, alone inhibited the ability of human prostate cancer cells to form colonies, invade, and migrate in vitro.<sup>66,67</sup> MnE also inhibited mouse breast cancer growth at high doses<sup>68</sup> and localized high concentrations of MnE on the skin dramatically inhibited skin cancer growth.<sup>69</sup> Thus, doses of MnP have to be very high to inhibit tumor growth alone. However, if applied along with additional, exogenous sources of H<sub>2</sub>O<sub>2</sub> such as radiation, chemotherapy, and ascorbate, the anticancer effects are greatly enhanced; MnPs are efficacious in the range of 0.2-2 mg/kg. Tumor growth inhibition with MnPs combined with radiation was observed in



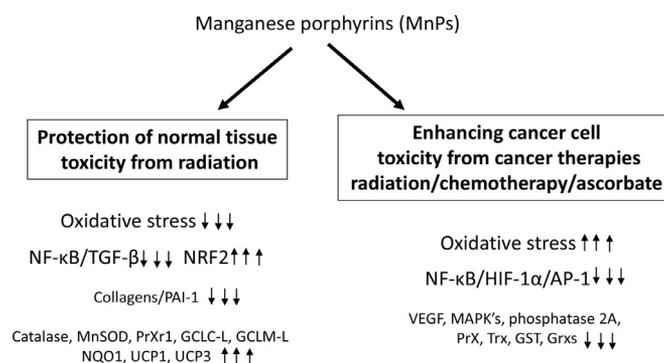
**Figure 3** The identified mechanisms by which MnPs protect normal tissues from radiation damage. MnPs inhibit inflammation by reducing the ability of NF-κB to bind to DNA through oxidation of the p50 subunit of NF-κB; the effect upon p65 is also likely. TGF-β signaling is reduced by MnP treatment through the reduction of active TGF-β, reduction in expression of TGFβRII, and a reduction in the phosphorylation of SMAD2 and SMAD3. These changes result in a reduction of SMAD complexes binding to DNA and transcribing profibrotic genes. MnP treatment enhances binding to antioxidant response element (ARE) to enhance antioxidant and cytoprotective gene expression.

several studies on glioma, head and neck, prostate, and breast cancer rodent studies.<sup>42,55,57,66,70</sup> MnBuOE promoted the anticancer effect of temozolomide in brain tumors<sup>57,58</sup> and both MnBuOE and MnE sensitize breast tumors to radiation and ascorbate.<sup>71,72</sup>

The mechanisms involved in cancer inhibition are less straightforward as compared to the mechanisms involved in protection of normal tissue injury. Two things are essential when considering a mechanism on the molecular level: (1) we need to view cancer as an aggravated oxidative stress injury with inflammation; and (2) the MnP has no way to

distinguish normal from tumor tissue other than differentiating between their redox environments. Cancer tissues have higher H<sub>2</sub>O<sub>2</sub> and MnP levels as compared to normal tissues.<sup>42,72</sup> When the normal tissues become highly oxidatively stressed and, thus, contain high H<sub>2</sub>O<sub>2</sub> levels, the MnP would increase injury in the normal tissues.<sup>52</sup> Conversely, as a single agent, with no additional H<sub>2</sub>O<sub>2</sub> added exogenously, MnP inflicts little damage to the cancer cell.<sup>42,68</sup>

MnE affects the activity of several transcription factors that promote tumor progression (Fig. 4). Radiation causes tumor hypoxia, which promotes HIF-1α, to enhance VEGF



**Figure 4** MnP affects signaling differently in normal vs cancer cells undergoing cancer therapy. In normal tissues, there is an overall reduction in oxidative stress when treated with MnPs and the NF-κB and TGF-β signaling pathways are reduced, while Nrf2 pathways are enhanced. In cancer cells, MnP treatment in combination with cancer therapy results in more oxidative stress, which results in reduction of NF-κB and HIF-1α and their downstream signaling pathways. These changes in signaling have been shown to enhance apoptosis of cancer cells and reduce overall cancer growth.

expression and revascularize the tumor.<sup>73</sup> MnE inhibited HIF-1α activity in hypoxic areas of the tumor after irradiation.<sup>74</sup> AP-1 controls genes involved in proliferation of skin cancer cells and MnE inhibited skin tumor growth, in part, by inactivating AP-1.<sup>69</sup> NF-κB activity was inhibited in lymphoma cells treated with MnP through S-glutathionylation of cysteines on the NF-κB p65 subunit, which promoted apoptosis.<sup>75,76</sup> Subsequently, redox proteomics performed on breast cancer cells treated with MnP/ascorbate, confirmed protein S-glutathionylation of the NF-κB pathway, p38MAPK, p38α (MAPK14), TAB3 and HSP60, phosphatase 2A pathway and endogenous antioxidant defenses, including peroxiredoxins and thioredoxins.<sup>42</sup> These data are supported by a recent breast cancer study where MnHex suppressed the phosphorylation of several MAPK targets: AKT, p38, ERK, and JNK.<sup>70</sup> These changes in signaling increased oxidative stress and caused the cancer cells to undergo apoptosis.<sup>42</sup>

## Clinical Trials of Mn Porphyrins as Radioprotectors

MnBuOE was selected for clinical trials as a radioprotector in cancer patients due to high biodistribution in all tissues, including brain, favorable safety/toxicity properties, and a wealth of preclinical data. In the first Phase I clinical trial, MnBuOE was tested as a radioprotector of normal brain tissues in glioma patients (NCT02655601). MnBuOE has passed the phase I trial, using 4 escalating doses, with no adverse events. This drug is currently entering Phase II trials. MnBuOE is also in Phase I clinical trials as a radioprotector of normal tissues for head and neck cancer and anal cancer patients as well as radio protection of brain in patients carrying multiple brain metastases, NCT03608020.

## Conclusions

We are fortunate to witness representatives of 2 different classes of metal complexes with SOD activity reaching

clinical trials at the same time—GC4419 (enantiomer of GC4403) and MnTnBuOE-2-PyP (MnBuOE or BMX-001). Moreover, these compounds are being tested in the same clinical application, radioprotection of normal tissue with head and neck cancer patients. The reason for such coincidence is the: (1) lack of drugs for the protection of normal tissue from radiation; (2) awareness of the key role of redox signaling pathways in cell biology, and (3) wealth of preclinical data indicating the impact of such compounds on redox signaling pathways in both radioprotection and cancer studies. Both compounds were originally developed as potent SOD mimics. Over time, data has emerged to show that these compounds, directly or indirectly, target several redox-sensitive pathways, which results in the restoration of the cellular physiological redox environment.

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